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# Hepatitis D Virus Genotypes in Intravenous Drug Users in Taiwan: Decreasing Prevalence and Lack of Correlation with Hepatitis B Virus Genotypes

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**Of 368 hepatitis B virus (HBV)-infected intravenous drug users, 144 (39%) were positive for antibody to hepatitis D virus (anti-HDV). Anti-HDV-positive HBV carriers had a lower rate of positivity for HBV DNA than did anti-HDV-negative carriers (52 versus 73%, respectively). From 1986 to 1997, the average rate of decrease in the prevalence of HDV infection in this population was 4.7%/year.**

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people in the world are chronic carriers of the virus (15). The infection can cause acute and chronic hepatitis, cirrhosis, and even hepatocellular carcinoma (3, 15). Currently, seven HBV genotypes (A to G) are identified based on the comparison of complete genomes (25, 30), and most of the genotypes have distinct geographical distributions (24, 25, 30). Genotypes B and C are prevalent in Asia, whereas genotypes A and D prevail in Western countries. Genotype E is restricted to Africa, and genotype F is found in Central America. Genotype G has been identified in France and North America only very recently (30). Although the clinical significance of HBV genotypes remains to be firmly settled, it has been shown elsewhere that genotype C is associated with the development of cirrhosis and hepatocellular carcinoma (7, 18, 28) and has a lower rate of response to interferon therapy than does genotype B (17). In addition, although superinfection with HBV indeed exists, it is rarely associated with acute exacerbations of chronic hepatitis B (19).

Hepatitis D virus (HDV), a defective RNA virus that requires the provision of hepatitis B surface antigen (HBsAg) from HBV for packaging and transmission (21), plays an important role in fulminant hepatitis and the progression of chronic liver damage in patients with chronic hepatitis B (2, 9, 10). HDV is currently classified into three genotypes (I to III) based on sequence comparison (2, 29, 34). Genotype I is the most common and widely distributed in Western Europe, North Africa, the Middle East, and East Asia. In contrast, genotypes II and III have a much more restricted distribution. Genotype II has been isolated from patients from Japan and Taiwan, where it may coexist with genotype I (33). Genotype III appears to be localized in northern South America (2). Studies of the relationship between HDV genotype and severity of disease are limited, although genotype II has been reported elsewhere to be less pathogenic than genotype I (33). On the other hand, genotype III is frequently associated with fulminant hepatitis (1). Taking these lines of evidence together, the determination of HBV and HDV genotypes may have both epidemiologic and clinical implications.

In Taiwan, an area where HBV infection is hyperendemic (3), HDV infection is generally infrequent in asymptomatic HBsAg carriers and patients with HBsAg-positive chronic liver diseases (5, 6). Nevertheless, the prevalence of antibody to HDV (anti-HDV) in HBsAg-positive intravenous drug users was extremely high as shown in our previous studies (6, 12). Intravenous drug users generally carry an extremely high risk of hepatitis C virus (HCV) infection, with the prevalence of antibody to HCV (anti-HCV) ranging from 70 to 90% in different parts of the world (16). It has been suggested elsewhere that the different distribution patterns of HDV genotypes may reflect the interaction between the HDV genotypes and dominant HBV genotypes in specific geographic areas (1); however, it is not known whether such an interaction holds true in Taiwan, where different HBV and HDV genotypes coexist. In the present study, the prevalence of HBV, HCV, and HDV infection was investigated in a correction center for illicit drug users for 1,651 inmates. The HBV genotypes were further determined in a cohort of HBsAg-positive intravenous drug users, with special reference to the distribution of HDV genotypes in those with HDV coinfection.

In January 1997, 1,651 (91%) serum samples from 1,815 men were collected from a correction center for illicit intravenous drug users in Penghu County, Pescadores, located west of Taiwan. The inmates were from all over the country, and the ages ranged from 20 to 67 years, with a mean of 35. All of them had intravenous drug use as the charge for current imprisonment. The study was initiated by the Center for Disease Control of the Department of Health, Taiwan, and informed consent was obtained from each inmate who participated in the study.

HBsAg, anti-HCV, and anti-HDV were tested with commercially available kits (Ausria II, HCV EIA II, and Anti-Delta, respectively; Abbott Laboratories, North Chicago, Ill.). HBV genotypes were determined by using PCR-restriction fragment length polymorphism of the surface gene of HBV as previously

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TABLE 1. Distribution of HDV genotypes in 31 HBsAg-positive intravenous drug users with HBV genotype B or C infection

| HBV genotype (no.) | No. (%) of subjects infected with HDV genotype(s): | | |
|---|---|---|---|
| | I | II | I + II |
| B (23) | 8 (35) | 13 (57) | 2 (8) |
| C (8) | 3 (37) | 5 (63) | 0 |

described (26). Six genotypes (A to F) of HBV could be identified by the restriction patterns of DNA fragments. HBV DNA transcripts with known amounts ($10^8$ copies/ml) were 10-fold serially diluted and used to determine the sensitivity of this genotyping assay. The sensitivity of the in-house PCR assay in our hands reached 10 copies of HBV DNA. HDV genotypes were also determined by using the PCR-restriction fragment length polymorphism method, and three genotypes (I to III) could be identified by the restriction patterns of DNA fragments as previously described (33). To avoid false-positive results, instructions to prevent cross contamination were strictly followed (20), and results were considered valid only when they were consistently obtained in duplicate.

Fisher's exact test and the chi-square test with Yates' correction were used where appropriate. A $P$ value of $<0.05$ was considered statistically significant.

Of 1,651 intravenous drug users, HBsAg and anti-HCV were detected in 368 (22.3%) and 983 (59.5%), respectively. Of the 368 HBsAg carriers, 144 (39%) were positive for anti-HDV and 240 (65%) were positive for serum HBV DNA by an in-house sensitive PCR assay (26). The genotype distribution of HBV was as follows: A, 8 (3.3%); B, 194 (80.8%); C, 37 (15.5%); and D, 1 (0.4%). No genotype E or F was found. Accordingly, genotype B strains were the predominant strains among these intravenous drug users persistently infected with HBV. Among these 144 anti-HDV-positive HBV carriers, 76 (52%) were positive for serum HBV DNA. In contrast, 164 (73%) of 224 anti-HDV-negative HBV carriers were positive for serum HBV DNA. The difference was statistically significant (52 versus 73%, $P < 0.001$). In addition, anti-HDV-positive HBV carriers were older than anti-HDV-negative carriers ($38.0 \pm 6.9$ versus $32.9 \pm 7.4$ years, respectively; $P < 0.001$).

Of 144 anti-HDV-positive HBsAg carriers, 31 (21.5%) were positive for serum HDV RNA, and the HDV genotype distribution was as follows: I, 11 (35.5%); II, 18 (58.1%); and mixed infection of I and II, 2 (6.4%). No genotype III was found. Thus, genotype II strains were the predominant strains among these HBV carriers with HDV coinfection. We could not find any particular HDV genotype linked to HBV genotype B or C infection (Table 1).

Intravenous drug users are at risk of multiple blood-borne viral infections such as those with HBV, HDV, HCV, and human immunodeficiency virus worldwide. In contrast to the intravenous drug users in Western countries, those in Taiwan are not yet contaminated by human immunodeficiency virus, as evidenced by seronegativity in this population (23). Previous epidemiologic surveys have indicated that the prevalence of HBsAg and anti-HCV in Taiwanese intravenous drug users was 19 and 81%, respectively (4, 6, 12), and the presence of anti-HDV in drug users who were asymptomatic HBsAg carriers ranged from 91% in 1986 to 85% in 1988 (6, 12).

In the present study, we reexamined the prevalence of HBV, HCV, and HDV infection in a large series of intravenous drug users 11 years later, and our results showed that the seroprevalence of HBsAg and anti-HCV was 22.3 and 59.5%, respectively. In addition, the positivity for anti-HDV in HBsAg-positive drug users was 39%. These data suggest that in Taiwan the circulation of HCV and HDV infection in this at-risk population has greatly diminished in the past decade. We estimate that from 1986 (12) to 1997, the rate of decrease in the proportion of intravenous drug users with HCV infection and HBsAg carrier drug users with HDV infection was about 1.9 and 4.7%/year, respectively. The decline in HCV and HDV infection may be a consequence of active preventive measures directed against promiscuity and sexually transmitted disease and the promotion of disposable needles to combat the epidemic of AIDS (13), as has been observed in a recent Italian survey (8). Although the incidence of HCV and HDV infection is declining, the ongoing transmission of HCV and HDV among intravenous drug users may still occur even in the developed countries. Thus, it appears that the only way to reduce HCV and HDV infection in this special population is by eliminating the opportunities for equipment sharing. However, this goal may require a deeper understanding of the social and behavioral determinants of sharing (14). By contrast, the prevalence of HBsAg in this study was similar to that of previous studies (19 versus 22%). These findings are not unanticipated, because most HBsAg carriers in Taiwan contracted HBV infection during their perinatal periods or early childhood (3), and thus the modification of risky behaviors in adulthood will not change the prevalence of HBsAg carriage.

Acute HDV superinfection may suppress HBV replication and lead to subsequent clearance of hepatitis B e antigen (HBeAg) and even HBsAg (22). Our data consistently showed a much lower rate of HBV DNA positivity in serum by PCR assays among the population previously ever infected with HDV than among those negative for anti-HDV (52 versus 73%, respectively; $P < 0.001$), suggesting that previous HDV infection indeed effectively suppresses the replication of HBV in HBsAg carriers. However, the possibility that the decreased replication of HBV after a longer duration of infection as reflected by the greater age of our anti-HDV-positive HBV carriers cannot be excluded. Consistent with a previous report (32), our results showed that serum HDV RNA was detectable in only 22% of asymptomatic HDV-infected subjects. These HDV RNA-negative, anti-HDV-positive HBV carriers may therefore have had a prior infection with HDV. Mixed HDV infections, though rare, have been documented previously (31). Thus, mixed infection with HDV genotypes I and II in 6% of our anti-HDV-positive HBsAg carriers was not surprising, since intravenous drug users are at a greater risk of multiple exposures to different HDVs. Because of the limitations of amplification technology, the present HBV genotyping method can detect only the dominant HBV genotype unless the titer of each genotype is comparable. Accordingly, the prevalence of mixed infections with different HBV genotypes may have been underestimated in the present study.

Superinfection with HDV in chronic HBV carriers has previously been reported to aggravate frequently the clinical

course of chronic hepatitis B in Western countries (11). Although HBsAg carriers with intravenous drug abuse in Taiwan are commonly infected with HDV, the infection does not seem to affect the liver disease (12). The less aggressive course of HDV infection in Taiwan than in Western countries may be explained by the different genotypes of HDV in specific geographic areas. Genotype II has been shown to be the predominant HDV genotype in Taiwan, which is linked to milder disease. In contrast, genotype I is the only isolate currently found in Western countries, and the disease severity in patients with genotype I infection seems to vary from mild to significant (33). Our data consistently showed that the predominant HBV and HDV genotypes in Taiwan were genotype B and genotype II, respectively (18, 33). Although specific interactions have been described elsewhere for HDV genotype III and HBV genotype F in the Peruvian Amazon basin (1), no particular HDV genotype was linked to HBV genotype B or C infection in Taiwan (Table 1).

In Taiwan, a national anti-hepatitis B vaccination of infants has been in effect since 1984, and the HBsAg carrier rate in children has decreased dramatically from 9.8 to 0.7% 15 years after the beginning of immunization (27). The decreased prevalence of HBV infection is depleting the HBsAg carrier reservoir, therefore decreasing the number of subjects susceptible to HDV infection and depriving this defective virus of the biological substrate necessary for its survival. Collectively, the declining prevalence of both HBV and HDV infection may herald complete control of HDV infection in Taiwan in the near future.

In summary, our findings indicate a declining prevalence of HCV and HDV infection among intravenous drug users in Taiwan. HBV genotype B and HDV genotype II are the major types of viral strains in this special population; however, no particular HDV genotype is linked to specific HBV genotype infection.

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